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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Dicamba - Acute Neurotoxicity Study in Rats

TO: Jane Mitchell/Walter Waldrop PM 71
SRRD (H7508W)

FROM: K. Clark Swentzel K. Clark Swentzel 11/3/93
Section Head, Section 2
Toxicology Branch II
HED (H7509C)

THROUGH: Marcia van Gemert, Ph.D. Marcia van Gemert 11/3/93
Branch Chief
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CASE: 818698
BARCODE: D192303
MRID 427741-04
SUBMISSION: S424717
PC NO. 029801
CASWELL NO. 295
REGISTRANT: Sandoz Inc.

Requested Action

Review attached submitted in response to DCI for Dicamba acid to fulfill acute neurotoxicity requirements.

Response

The subject study has been reviewed by Clement International Corp.; the DER is attached.

Conclusions

A single dose of Dicamba was administered by gavage to Crl:CD BR rats at doses of 0, 300, 600 or 1200 mg/kg. Control rats received vehicle (corn oil) only. Positive controls received acrylamide (50 mg/kg/day) by intraperitoneal injection on 7 consecutive days.

NOEL < 300 mg/kg (low dose)

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LOEL = 300 mg/kg based on transiently impaired respiration, rigidity upon handling, prodding or dropping, freezing of movement when touched, decreased arousal and fewer rears/minute compared to controls, impairment of gate and righting reflex in both sexes. In addition, males showed decreased forelimb strength, which persisted until day 7, these effects were observed only on the day of dosing.

At 600 mg/kg, both sexes showed decreases in locomotor activity and males showed significant decreases in tail flick reflex and a raised posture when placed in an open field. These effects were also observed on the day of dosing.

At 1200 mg/kg, both sexes showed an impaired startle response to an auditory stimulus. The effect was significant in males on day 7 and in females on the day of dosing. In addition, males showed decreases in body weight (5-9%), in body weight gain (24%) and food consumption (13%) between days 0 and 7.

Core classification

Minimum: This study satisfies the guideline requirements for an acute neurotoxicity study in rats (81-8).

cc: Jess Rowland

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FINAL

DATA EVALUATION REPORT

Dicamba

Study Type: Acute Neurotoxicity Screening Battery

Prepared for:

Office of Pesticide Programs
Health Effects Division
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
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Fairfax, VA 22031-1207

October 22, 1993

Principal Reviewer _____ Date 10/21/93
Carrie Rabe, Ph.D.

Independent Reviewer _____ Date 10/21/93
John Liccione, Ph.D.

QA/QC Manager _____ Date 10/21/93
Sharon Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 2-137
Clement Number: 472
Project Officer: Caroline Gordon

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EPA Reviewer and Section Head:
Clark Swentzel, Review Section II,
Toxicology Branch II, Health Effects Division

Signature: K. Clark Swentzel
Date: 11/3/93

DATA EVALUATION REPORT

STUDY TYPE: Acute oral neurotoxicity screening battery in rats (Guideline series 81-8)

TEST MATERIAL: Dicamba

TOX. CHEM. NUMBER: 295 P.C. NUMBER: 029801

CAS NUMBER: 1918-00-9

SYNONYMS: None reported

STUDY NUMBER: HWA 686-177 MRID NUMBER: 427741-04

SPONSORS: Sandoz Agro, Inc.
Des Plaines, Illinois

TESTING FACILITY: Hazleton Washington, Inc.
Vienna, Virginia

TITLE OF REPORT: Acute Neurotoxicity Study of Technical Dicamba by Gavage in Rats

AUTHOR: D.J. Minnema

REPORT ISSUED: Study completed May 11, 1993

QUALITY ASSURANCE: A signed Good Laboratory Practice Compliance Statement, a signed Quality Assurance Statement, and a list of Quality Assurance Inspections were included.

CONCLUSIONS: Dicamba was administered by gavage in a single dose to Crl:CD BR rats at doses of 0, 300, 600, or 1,200 mg/kg. Rats at 0 mg/kg received vehicle (corn oil) only. Positive controls received acrylamide (50 mg/kg/day) by intraperitonaal injection on seven consecutive days.

NOEL < 300 mg/kg

LOEL = 300 mg/kg based on transiently impaired respiration, rigidity upon handling, prodding, or dropping, freezing of movement when touched, decreased arousal and fewer rears/minute compared to controls, impairment of gait and righting reflex in both sexes. In addition, males showed decreased forelimb grip strength. With the exception of the decrease in forelimb grip strength, which persisted until day 7, these effects were observed only on the day of dosing.

In addition, at 600 mg/kg, both sexes showed decreases in locomotor activity and males showed significant decreases in tail flick reflex and a raised posture when placed in an open field. These effects were also observed only on the day of dosing.

At the highest dose tested, both males and females showed an impaired startle response to an auditory stimulus. The effect was significant in males on day 7 and in females on the day of dosing. In addition, males showed decreases in body weight (5-9%) and in body weight gain (24%) and food consumption (11%) between days 0 and 7.

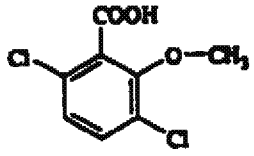
CORE CLASSIFICATION: Core Minimum. This study satisfies the guideline requirements for an acute neurotoxicity study and is classified as Core Minimum because a NOEL was not determined. Also, no verification of the concentration of the test material was provided.

A. MATERIALS, METHODS, AND RESULTS

1. Test Article Description

Name: Technical dicamba

Formula: 3,6-dichloro-2-methoxybenzoic acid



Lot number: Batch 52103810

Purity: 86.9%; impurities not reported

Physical property: Beige flakes

Srability: Not reported

Storage: Stored in a cool, dry place

2. Rationale for Dose Selection

The selection of doses used in this study was reported to be based on the results of a preliminary time-course study (#686-179). The results of that study were not specified.

3. Test Article Preparation and Analyses for Purity and Stability

The purity and stability of the test material were not verified by the testing facility. However, the sponsor identified the purity of the batches sent to the testing facility as 86.9% pure (batch 52103810).

The test material was prepared for oral gavage dosing by grinding the flakes into a powder, preparing a paste by mixing a small amount of

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vehicle (corn oil) with the powder, and adding additional vehicle to bring the suspension to the desired concentration. Dose preparation assumed 100% purity of the test material. Stirring was continued until the test material had gone into solution. Verification of achievement of the desired concentrations was not provided. The positive control solutions were prepared by mixing acrylamide (assumed purity of 100%) with a 0.9% sodium chloride solution.

4. Animals

Crl:CD BR rats (63 males and 63 females) were received from Charles River Laboratories, Inc., Raleigh, North Carolina. The rats were approximately 4 weeks old upon arrival and were housed 2/cage (same sex) in stainless-steel wire mesh cages. The animal room was operated on a 12-hour light/dark cycle, and temperature and relative humidity ranged between 70.2°F and 76.0°F and between 28.8% and 69.7%, respectively. Feed (Purina Certified Rodent Chow #5002) and water were available ad libitum.

Fifty rats of each sex were randomly allocated (10/sex/dose) using a computerized random number generation system to five treatment groups after removal of animals with clinical signs or extreme body weights.

Group	Number of Animals	
	Males	Females
Vehicle control (corn oil)	10	10
300 mg/kg	10	10
600 mg/kg	10	10
1,200 mg/kg	10	10
Positive control (acrylamide, 50 mg/kg)	10	10

Treatment groups were selected such that the mean body weights of each group were not significantly different. At the time of the first exposure to the test material, the rats were approximately 7 weeks old, and males and females ranged in weight from 216 to 263 g and from 159 to 208 g, respectively. The rats were uniquely identified with implanted microchips. Animals were exposed to a reversed light/dark cycle (i.e., light from 8 p.m. until 8 a.m., dark from 8 a.m. to 8 p.m.).

5. Dosing Regimen

The vehicle control and test material were administered as a single gavage dose (5 mL/kg). The positive control (acrylamide, 50 mg/kg) was administered by intraperitoneal injection (1 mL/kg) once daily for 7 consecutive days. The vehicle control and test material solutions were coded so that the laboratory personnel would not know which dose the animals received.

6. Statistical Analyses

Body weight, body weight gain, and food consumption were analyzed using a one-way analysis of variance. Prior to the analysis of variance, Levene's test was used to assess homogeneity of variances. If variances were heterogeneous, data were transformed (rank transformations) to achieve homogeneity. Analysis of variance was then performed. If the result was significant ($p \leq 0.05$), Dunnett's test was used to analyze for differences between the control and treated groups.

Behavioral data (step latency, number of rears, number of urine pools, number of fecal boli, startle data, tail flick latency, fore- and hindlimb grip strength, landing foot splay, and total activity counts (in 5 minute blocks) were analyzed by a factorial analysis of variance with repeated measures. If the result was significant (by dose or time), univariate analysis of doses to control was performed.

7. General Observations

(a) Mortality/moribundity/survival

Animals were observed twice daily for mortality/moribundity.

One male at 1,200 mg/kg was found dead on the day after dosing. It is probable that this death was treatment-related. Necropsy of this animal revealed dark red lungs.

(b) Clinical observations

Animals were observed once daily for overt adverse clinical signs. In addition, detailed physical examinations for adverse clinical signs were made weekly.

No overt signs of toxicity were observed.

(c) Body weights/food consumption

Body weights--Individual body weights were determined prior to dosing and at days 7 and 14.

Statistically significant decreases in body weight were observed in males at 1,200 mg/kg and in both males and females in the positive control group at the day-7 and day-14 weighings (Table 1).

Mean body weight gains of males at 1,200 mg/kg were significantly decreased over the interval of days 0-7. Both males and females in the positive control groups showed significant decreases in body weight gain over the intervals of days 0-7 and 0-14 (Table 1).

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Table 1. Mean Body Weight and Body Weight Gain for Rats Given Technical Dicamba by Gavage[a,b,c]

	Mean Body Weight (g ± S.D.) by Dose Level (mg/kg)				
Day	0	300	600	1200	Positive control
			Males		
0	240.2 ± 14.4	231.2 ± 10.8 (96)	233.3 ± 10.3 (97)	229.3 ± 7.2 (95)	235.7 ± 11.3 (98)
7	296.9 ± 19.4	282.7 ± 17.6 (95)	282.1 ± 16.0 (95)	271.3 ± 12.2** (91)	254.1 ± 9.3** (86)
14	318.6 ± 23.6	334.6 ± 21.4 (96)	339.1 ± 19.8 (97)	323.7 ± 15.9* (93)	312.6 ± 12.7** (90)
			Females		
0	180.7 ± 9.4	181.0 ± 8.2 (100)	174.3 ± 11.8 (96)	182.2 ± 11.2 (101)	175.2 ± 11.8 (97)
7	202.8 ± 15.4	204.2 ± 10.2 (101)	193.4 ± 16.3 (95)	203.2 ± 12.9 (100)	175.0 ± 16.8** (86)
14	224.2 ± 16.7	230.3 ± 15.1 (103)	217.9 ± 17.0 (97)	232.2 ± 20.9 (104)	198.4 ± 17.1** (88)

	Mean Body Weight Gain (g ± S.D.) by Dose Level (mg/kg)				
Days	0	300	600	1200	Positive control
			Males		
0-7	56.7 ± 7.1	51.5 ± 7.8 (91)	48.8 ± 7.5 (86)	43.3 ± 7.3** (76)	18.4 ± 8.9** (32)
7-14	51.7 ± 5.0	51.9 ± 5.5 (100)	57.0 ± 4.6 (110)	52.3 ± 8.2 (101)	58.5 ± 8.8 (113)
0-14	108.4 ± 11.5	103.4 ± 11.7 (95)	105.8 ± 11.4 (98)	95.7 ± 10.6 (88)	76.9 ± 7.7** (71)
			Females		
0-7	22.1 ± 8.2	23.2 ± 6.3 (105)	19.1 ± 6.3 (86)	21.0 ± 5.4 (95)	-0.2 ± 12.3** (0)
7-14	21.4 ± 6.9	26.1 ± 8.4 (122)	24.5 ± 5.3 (114)	29.0 ± 13.5 (136)	23.4 ± 10.4 (109)
0-14	43.5 ± 11.8	49.3 ± 10.5 (113)	43.6 ± 7.1 (100)	50.0 ± 17.1 (115)	23.2 ± 10.5** (53)

[a] Data extracted from Study MRA 686-177, Tables 2A and 2B
[b] Numbers in parentheses indicate percent of control
[c] N = 10 for all groups except the 1200-mg/kg males for which N = 9 on days 7 and 14, and over the periods of days 0-7, 7-14, and 0-14.

* Significantly different from control; $p \leq 0.05$
** Significantly different from control; $p \leq 0.01$

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Food consumption--Individual food consumption values were determined weekly.

Mean total weekly food consumption was significantly decreased in males at 1,200 mg/kg during the first week of the study (Table 2). Mean total weekly food consumption values in males and females in the vehicle control group were also decreased during this interval.

8. Functional observational battery

Animals were evaluated based on the following end points prior to dosing, within 0.5-2.5 hours of dosing, and on days 7 and 14. All observations except performance evaluations were conducted in darkened rooms with red light illumination. Following the home cage observations, the animals were moved to an enclosed area measuring 66 (length) x 48 (width) x 30.5 (height) cm and were observed for 1 minute to determine the presence of the pen field parameters. Response observations were also measured in this enclosed area. Detailed descriptions of the response observations, performance measures, and startle response and locomotor activity measurements as well as criteria used to assess the behavioral end points are attached as Appendix 1.

Home Cage Observations

X Ease of removal from cage*
X Ease of handling/body tone*
X Palpebral closure*
X Color of tears/deposits around eyes*
X Respiration*
X Salivation*
X Appearance of fur*
X Convulsions/tremors*
X Piloerection*
X Writhing
X Excessive vocalization*
X Exophthalmus*
Posture/gait*
X Lacrimation*

Performance Measures

X Tail flick latency*
X Landing foot splay*
X Forelimb grip strength*
X Hindlimb grip strength*
X Rectal body temperature*

Open Field Observations

X Posture*
X Gait*
X Arousal*
X Circling*
X Stereotypy*
X Convulsions*
X Tremors*
X Urination*
X Defecation*
X Latency to first step
X Number of rears

Response Observations

X Approach response*
X Catalepsy withdrawal
X Righting reflex*
X Olfactory response
X Pupil response*
X Touch response*

X Locomotor activity*
X Automated startle response*

*Recommended by Subdivision F (March 1991) Guidelines

Several parameters assessed by the functional observational battery were affected by exposure to the dicamba (Tables 3-5).

Table 2. Mean Food Consumption for Rats Given Technical Dicamba by Gavage[a,b,c]

Days	Mean Food Consumption (g/rat ± S.D.) by Dose Level (mg/kg)				
	0	300	600	1200	Positive control
			Males		
0-7	184.3 ± 14.1	173.8 ± 15.9 (94)	174.7 ± 12.3 (95)	160.7 ± 11.2** (87)	141.8 ± 8.2** (77)
7-14	198.4 ± 24.4	190.0 ± 14.9 (96)	193.5 ± 14.6 (98)	182.9 ± 7.1 (92)	199.9 ± 16.4 (101)
			Females		
0-7	135.0 ± 12.4	130.6 ± 11.1 (97)	127.0 ± 10.0 (94)	127.0 ± 8.5 (94)	96.2 ± 10.9** (71)
7-14	149.5 ± 20.7	149.9 ± 22.0 (100)	141.4 ± 9.3 (95)	146.6 ± 19.6 (98)	137.8 ± 14.1 (92)

[a] Data extracted from Study MRA 686-177, Table 3
[b] Numbers in parentheses indicate percent of control
[c] N = 10 for all groups except the 1200-mg/kg males for which N = 9 for days 0-7 and N = 8 for days 7-14.

** Significantly different from control; $p \leq 0.01$

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Table 3a. Functional Observational Battery for Male Rats Given Technical Dicamba by Gavage[a,b]

Parameter	Incidence or Magnitude (mean ± S.D.) of Observation by Dose Level (mg/kg)				
	0	300	600	1200	Positive control
Handling/body tone–rigidity					
0.5-2.5 hr	0/10	0/10	8/10	8/10	0/10
7 d	0/10	0/10	0/10	0/9	0/10
14 d	0/10	0/10	0/10	0/9	0/10
Posture–raised					
0.5-2.5 hr	0/10	0/10	5/10	5/10	0/10
7 d	0/10	0/10	0/10	0/9	0/10
14 d	0/10	0/10	0/10	0/9	0/10
Gait–impaired					
0.5-2.5 hr	0/10	9/10 (1)[c]	10/10 (1.7)	10/10 (1.9)	0/10
7 d	0/10	0/10	0/10	0/9	10/10 (1)
14 d	0/10	0/10	0/10	0/9	10/10 (1)
Arousal–hypoalert					
pre-dose	0/10	0/10	0/10	1/10	0/10
0.5-2.5 hr	0/10	2/10	4/10	7/10	1/10
7 d	1/10	4/10	2/10	0/9	0/10
14 d	3/10	3/10	2/10	2/9	0/10
Rears (rears/min)					
pre-dose	5.5 ± 2.1	5.1 ± 2.1	6.5 ± 3.1	6.0 ± 3.4	5.2 ± 2.1
0.5-2.5 hr	4.4 ± 1.6	2.1 ± 1.8*	1.7 ± 1.4*	0.7 ± 0.7*	3.1 ± 2.0
7 d	1.6 ± 2.2	1.1 ± 1.4	1.3 ± 2.2	2.1 ± 2.6	0.6 ± 1.0
14 d	1.8 ± 1.7	1.5 ± 2.5	2.5 ± 3.5	3.1 ± 3.0	1.0 ± 1.4
Touch response–freezes when touched					
0.5-2.5 hr	0/10	1/10	5/10	4/10	0/10
7 d	0/10	0/10	0/10	0/9	0/10
14 d	0/10	0/10	0/10	0/9	0/10
Tail flick latency (sec)					
pre-dose	7.7 ± 5.0	9.0 ± 6.2	7.1 ± 5.1	10.5 ± 5.1	10.2 ± 4.4
0.5-2.5 hr	11.1 ± 4.7	13.7 ± 3.8	17.1 ± 4.7*	20.7 ± 5.4*	13.2 ± 3.0
7 d	13.3 ± 3.9	14.7 ± 1.6	12.7 ± 3.7	14.8 ± 1.9	12.9 ± 2.5
14 d	15.5 ± 2.0	13.8 ± 3.9	14.1 ± 2.2	14.1 ± 2.0	14.2 ± 3.5
Forelimb grip strength (g)					
pre-dose	713 ± 157	721 ± 78	659 ± 96	685 ± 111	665 ± 77
0.5-2.5 hr	986 ± 145	836 ± 148*	803 ± 150*	698 ± 61*	900 ± 133
7 d	1228 ± 121	1111 ± 175	1204 ± 139	1043 ± 118*	1061 ± 113*
14 d	1239 ± 138	1190 ± 149	1269 ± 208	1183 ± 172	1110 ± 263
Righting reflex–uncoordinated					
pre-dose	0/10	0/10	2/10	1/10	1/10
0.5-2.5 hr	2/10	4/10	7/10	1/10	3/10
7 d	1/10	0/10	2/10	0/9	1/10
14 d	0/10	1/10	0/10	0/9	0/10
Righting reflex–lands on side					
0.5-2.5 hr	0/10	2/10	3/10	7/10	0/10
7 d	0/10	0/10	0/10	0/9	0/10
14 d	0/10	0/10	0/10	0/9	0/10
Righting reflex–lands on back					
0.5-2.5 hr	0/10	1/10	0/10	1/10	0/10
7 d	0/10	0/10	0/10	0/9	0/10
14 d	0/10	0/10	0/10	0/9	0/10

Table 3a (continued)

Parameter	Incidence or Magnitude (mean ± S.D.) of Observation by Dose Level (mg/kg)				
	0	300	600	1200	Positive control
Respiration-impaired					
0.5-2.5 hr	0/10	1/10 (1)[c]	2/10 (1)	4/10 (1.25)	0/10
7 d	0/10	0/10	0/10	0/9	6/10 (1)
14 d	0/10	0/10	0/10	0/9	3/10 (1)
Other Signs					
pre-dose	0/10	1/10[d]	0/10	0/10	0/10
0.5-2.5 hr	0/10	6/10[e]	9/10[e]	8/10[d,e]	0/10
7 d	0/10	0/10	0/10	0/9	1/10[f]
14 d	0/10	0/10	0/10	0/9	0/10
Hindlimb grip strength (g)					
pre-dose	318 ± 110	348 ± 69	332 ± 64	345 ± 76	311 ± 67
0.5-2.5 hr	554 ± 87	584 ± 63	515 ± 71	436 ± 72	483 ± 73
7 d	813 ± 127	746 ± 72	729 ± 138	707 ± 112	669 ± 143
14 d	939 ± 120	914 ± 110	879 ± 110	832 ± 129	735 ± 137

[a] Data extracted from Study MRA 686-177, Tables 4B, 4G, 5B, 5C, 5I, 5N, 6B, 6F, 6G, 7A, 7C, and 7D
[b] N = 10 for all groups except the 1200-mg/kg males for which N = 9 on days 7 and 14.
[c] Values in parentheses indicate degree of impairment (0 = normal, 1 = slightly impaired, 2 = moderately impaired, and 3 = severely impaired).
[d] One animal bit cotton swab during olfactory response test.
[e] Animal became rigid upon dropping (righting reflex) and in some cases, touch response.
[f] Repeated muscle contractions of dorsal surface.

* Significantly different from controls; $p \leq 0.05$

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Table 3b. Functional Observational Battery for Female Rats Given Technical Dicamba by Gavage[a,b]

Parameter	Incidence or Magnitude (mean ± S.D.) of Observation by Dose Level (mg/kg)				
	0	300	600	1200	Positive control
Handling/body tone–rigidity					
0.5-2.5 hr	0/10	5/10	8/10	10/10	0/10
7 d	0/10	0/10	0/10	0/10	0/10
14 d	0/10	0/10	0/10	0/10	0/10
Posture–raised					
0.5-2.5 hr	0/10	2/10	6/10	6/10	0/10
7 d	0/10	0/10	0/10	0/10	1/10
14 d	0/10	0/10	0/10	0/10	1/10
Gait–impaired					
0.5-2.5 hr	0/10	10/10 (1)[c]	10/10 (1.4)	10/10 (1.9)	0/10
7 d	0/10	0/10	0/10	0/10	10/10 (1.1)
14 d	0/10	0/10	0/10	0/10	10/10 (1.3)
Arousal–hypoalert					
0.5-2.5 hr	1/10	2/10	2/10	0/10	0/10
7 d	0/10	0/10	0/10	1/10	1/10
14 d	1/10	0/10	1/10	0/10	1/10
Rears (rears/min)					
pre-dose	6.2 ± 2.4	8.2 ± 3.7	8.1 ± 1.6	6.4 ± 3.3	6.2 ± 2.0
0.5-2.5 hr	5.0 ± 4.0	3.2 ± 2.2	2.7 ± 2.7	2.0 ± 1.2	4.0 ± 2.0
7 d	7.2 ± 3.2	4.6 ± 3.0	5.7 ± 5.4	4.8 ± 2.9	0.4 ± 0.5*
14 d	5.9 ± 4.9	6.2 ± 3.7	7.3 ± 6.3	6.4 ± 2.2	2.2 ± 1.3
Touch response–freezes when touched					
0.5-2.5 hr	0/10	2/10	6/10	6/10	0/10
7 d	0/10	0/10	0/10	0/10	0/10
14 d	0/10	0/10	0/10	0/10	0/10
Tail flick latency (sec)					
pre-dose	10.3 ± 4.8	10.0 ± 4.1	10.2 ± 3.6	11.4 ± 3.5	10.6 ± 4.1
0.5-2.5 hr	14.6 ± 4.7	14.8 ± 6.5	14.7 ± 2.8	15.0 ± 1.9	11.0 ± 3.2
7 d	13.1 ± 5.4	12.4 ± 2.9	14.7 ± 4.1	12.7 ± 3.6	12.1 ± 2.6
14 d	13.5 ± 1.8	10.8 ± 4.0	12.7 ± 4.2	12.6 ± 2.7	13.0 ± 1.8
Forelimb Grip Strength (g)					
pre-dose	644 ± 115	691 ± 91	706 ± 85	719 ± 70	708 ± 133
0.5-2.5 hr	835 ± 159	829 ± 92	848 ± 179	746 ± 128	796 ± 152
7 d	1091 ± 94	1088 ± 90	1068 ± 112	1000 ± 165	801 ± 234
14 d	909 ± 316	939 ± 231	943 ± 226	996 ± 160	869 ± 156
Righting reflex–uncoordinated					
pre-dose	1/10	0/10	1/10	0/10	0/10
0.5-2.5 hr	0/10	6/10	1/10	1/10	0/10
7 d	0/10	0/10	0/10	0/10	0/10
14 d	0/10	0/10	0/10	0/10	0/10
Righting reflex–lands on side					
0.5-2.5 hr	0/10	0/10	6/10	6/10	0/10
7 d	0/10	0/10	1/10	0/10	0/10
14 d	0/10	0/10	0/10	0/10	0/10
Righting reflex–lands on back					
0.5-2.5 hr	0/10	2/10	2/10	3/10	0/10
7 d	0/10	0/10	0/10	0/10	0/10
14 d	0/10	0/10	0/10	0/10	0/10

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Table 3b (continued)

Parameter	Incidence or Magnitude (mean ± S.D.) of Observation by Dose Level (mg/kg)				
	0	300	600	1200	Positive control
Respiration-impaired					
0.5-2.5 hr	0/10	0/10	1/10 (1)[c]	5/10 (1)	0/10
7 d	0/10	0/10	0/10	0/10	4/10 (1)
14 d	0/10	0/10	0/10	0/10	2/10 (1)
Other Signs					
0.5-2.5 hr	0/10	3/10[d]	9/10[d]	10/10[d]	0/10
7 d	0/10	0/10	1/10[e]	0/10	0/10
14 d	0/10	0/10	1/10[f]	0/10	0/10
Hindlimb grip strength (g)					
pre-dose	361 ± 68	338 ± 66	398 ± 88	330 ± 62	336 ± 77
0.5-2.5 hr	566 ± 85	550 ± 110	589 ± 92	505 ± 79	526 ± 92
7 d	726 ± 111	716 ± 74	788 ± 91	746 ± 116	513 ± 137*
14 d	866 ± 159	816 ± 101	865 ± 157	874 ± 141	589 ± 123*

[a] Data extracted from Study HWA 686-177, Tables 4B, 4G, 5B, 5C, 5I, 5N, 6B, 6F, 6G, 7A, 7C, and 7D
[b] N = 10
[c] Values in parentheses indicate degree of impairment (0 = normal, 1 = slightly impaired, 2 = moderately impaired, and 3 = severely impaired).
[d] Animal became rigid upon dropping (righting reflex) and in some cases, touch response.
[e] Repeated muscle contractions of dorsal surface.
[f] Animal tremored (briefly) immediately after dropping (righting reflex).

* Significantly different from controls; $p \leq 0.05$

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Table 4a. Locomotor Activity Data for Male Rats Given Technical Dicamba by Gavage[a,b]

Interval	Activity Counts (mean ± S.D.) by Dose Level (mg/kg)				
	0	300	600	1,200	Positive control
1-5 min					
pre-dose	463 ± 55	525 ± 86	487 ± 55	511 ± 78	546 ± 104
0.5-2.5 hr	386 ± 61	231 ± 192	101 ± 87*	48 ± 37*	344 ± 126
7d	346 ± 101	348 ± 167	351 ± 139	406 ± 82	310 ± 103
14d	387 ± 162	403 ± 184	406 ± 120	449 ± 144	380 ± 123
6-10 min					
pre-dose	295 ± 73	312 ± 95	297 ± 71	267 ± 54	320 ± 94
0.5-2.5 hr	239 ± 128	154 ± 115	45 ± 29*	14 ± 12*	159 ± 80
7d	282 ± 69	285 ± 154	290 ± 97	217 ± 72	232 ± 86
14d	283 ± 76	272 ± 171	286 ± 118	253 ± 104	235 ± 136
11-15 min					
pre-dose	172 ± 89	212 ± 81	182 ± 70	197 ± 79	221 ± 106
0.5-2.5 hr	122 ± 100	122 ± 76	34 ± 33	12 ± 10*	143 ± 111
7d	142 ± 107	216 ± 119	114 ± 64	177 ± 63	170 ± 114
14d	177 ± 126	190 ± 125	136 ± 82	181 ± 68	204 ± 91
16-20 min					
pre-dose	125 ± 106	143 ± 129	77 ± 97	166 ± 106	174 ± 116
0.5-2.5 hr	64 ± 98	53 ± 61	20 ± 29	11 ± 6	74 ± 76
7d	101 ± 62	177 ± 145	92 ± 91	90 ± 69	111 ± 111
14d	108 ± 85	151 ± 95	63 ± 100	90 ± 86	192 ± 99
21-25 min					
pre-dose	44 ± 79	68 ± 111	53 ± 83	104 ± 111	49 ± 62
0.5-2.5 hr	41 ± 70	23 ± 51	24 ± 25	5 ± 8	16 ± 22
7d	72 ± 73	81 ± 77	77 ± 99	78 ± 95	54 ± 65
14d	77 ± 104	141 ± 112	66 ± 81	45 ± 56	136 ± 94
26-30 min					
pre-dose	14 ± 30	12 ± 28	11 ± 17	34 ± 48	33 ± 66
0.5-2.5 hr	19 ± 30	16 ± 34	17 ± 29	24 ± 34	20 ± 33
7d	56 ± 93	51 ± 70	43 ± 75	63 ± 77	46 ± 51
14d	49 ± 67	27 ± 32	72 ± 102	87 ± 91	86 ± 85
31-35 min					
pre-dose	6 ± 7	23 ± 47	11 ± 22	24 ± 41	10 ± 16
0.5-2.5 hr	9 ± 16	36 ± 66	23 ± 44	11 ± 18	17 ± 37
7d	35 ± 59	65 ± 79	46 ± 76	40 ± 53	65 ± 91
14d	27 ± 34	15 ± 37	84 ± 84	66 ± 68	45 ± 85
36-40 min					
pre-dose	4 ± 3	7 ± 11	24 ± 57	15 ± 22	3 ± 3
0.5-2.5 hr	25 ± 76	3 ± 5	3 ± 3	27 ± 34	12 ± 26
7d	40 ± 81	21 ± 27	57 ± 93	55 ± 99	93 ± 106
14d	75 ± 99	36 ± 51	113 ± 104	80 ± 101	36 ± 56

[a] Data extracted from Study MRA 686-177, Table 9

[b] N = 10 for all groups except the 1,200 mg/kg males for which N = 9 on days 7 and 14.

* Significantly different from control; p ≤ 0.05

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Table 4b. Locomotor Activity Data for Female Rats Given Technical Dicamba by Gavage[a,b]

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Interval	Activity Counts (mean ± S.D.) by Dose Level (mg/kg)				
	0	300	600	1,200	Positive control
1-5 min					
pre-dose	473 ± 55	490 ± 69	476 ± 65	509 ± 39	506 ± 65
0.5-2.5 hr	447 ± 67	405 ± 102	321 ± 79*	167 ± 87*	455 ± 90
7d	423 ± 65	502 ± 85	461 ± 131	466 ± 94	376 ± 133
14d	469 ± 107	565 ± 37*	516 ± 62	561 ± 51	488 ± 99
6-10 min					
pre-dose	308 ± 51	376 ± 80	304 ± 86	296 ± 47	382 ± 76
0.5-2.5 hr	277 ± 44	204 ± 98	139 ± 98*	29 ± 30*	286 ± 61
7d	285 ± 81	351 ± 81	318 ± 84	274 ± 87	285 ± 143
14d	315 ± 91	322 ± 122	335 ± 73	342 ± 94	349 ± 128
11-15 min					
pre-dose	210 ± 87	296 ± 89	240 ± 32	165 ± 62	298 ± 135
0.5-2.5 hr	181 ± 81	161 ± 120	83 ± 55	21 ± 20*	213 ± 120
7d	170 ± 51	258 ± 110	198 ± 70	242 ± 96	178 ± 136
14d	156 ± 108	211 ± 86	179 ± 96	155 ± 57	244 ± 129
16-20 min					
pre-dose	170 ± 98	213 ± 105	133 ± 89	162 ± 118	169 ± 146
0.5-2.5 hr	111 ± 102	98 ± 104	70 ± 80	16 ± 16*	108 ± 80
7d	154 ± 72	207 ± 118	171 ± 97	159 ± 95	43 ± 53*
14d	100 ± 100	213 ± 151	121 ± 113	180 ± 126	181 ± 96
21-25 min					
pre-dose	96 ± 94	152 ± 117	66 ± 77	38 ± 70	175 ± 110
0.5-2.5 hr	54 ± 89	67 ± 87	51 ± 47	12 ± 11	65 ± 98
7d	99 ± 65	154 ± 116	8.. ± 92	176 ± 113	46 ± 88
14d	77 ± 106	150 ± 141	151 ± 117	120 ± 94	194 ± 101
26-30 min					
pre-dose	67 ± 93	66 ± 86	27 ± 57	28 ± 49	124 ± 130
0.5-2.5 hr	58 ± 87	41 ± 62	35 ± 44	11 ± 13	10 ± 18
7d	106 ± 102	79 ± 101	114 ± 113	146 ± 122	108 ± 149
14d	88 ± 121	80 ± 104	192 ± 102	113 ± 117	94 ± 88
31-35 min					
pre-dose	28 ± 75	90 ± 124	18 ± 30	72 ± 112	116 ± 114
0.5-2.5 hr	36 ± 83	21 ± 38	35 ± 50	18 ± 33	46 ± 98
7d	104 ± 116	72 ± 102	97 ± 102	108 ± 111	77 ± 102
14d	100 ± 117	128 ± 133	210 ± 139	165 ± 160	70 ± 88
36-40 min					
pre-dose	41 ± 67	63 ± 86	77 ± 120	61 ± 69	81 ± 86
0.5-2.5 hr	32 ± 76	43 ± 63	46 ± 56	26 ± 40	55 ± 84
7d	62 ± 72	95 ± 112	105 ± 113	92 ± 130	93 ± 145
14d	101 ± 99	115 ± 98	94 ± 94	85 ± 73	53 ± 113

[a] Data extracted from Study MRA 686-177, Table 9
[b] N=10

* Significantly different from control; p ≤ 0.05

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Table 5a. Auditory Response Data for Male Rats Given Technical Dicamba by Gavage[a,b]

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Parameter	Auditory Response Values (mean ± S.D.) by Dose Level (mg/kg)				
	0	300	600	1200	Positive control
Control-no stimulus					
Maximum amplitude of muscle response (mv)					
pre-dose	34 ± 25	46 ± 48	24 ± 13	24 ± 13	43 ± 30
0.5-2.5 hr	33 ± 27	19 ± 7	19 ± 14	11 ± 7*	14 ± 7
7 d	26 ± 16	22 ± 15	20 ± 7	12 ± 9	11 ± 15
14 d	24 ± 11	22 ± 17	17 ± 8	13 ± 9	8 ± 3*
Time to reach maximum amplitude (ms)					
pre-dose	54 ± 30	56 ± 29	26 ± 32	45 ± 26	51 ± 25
0.5-2.5 hr	51 ± 21	51 ± 35	35 ± 22	37 ± 25	57 ± 32
7 d	52 ± 37	38 ± 31	52 ± 25	26 ± 29	49 ± 36
14 d	38 ± 30	59 ± 31	48 ± 33	49 ± 29	50 ± 29
Average amplitude of muscle response (mv)					
pre-dose	10 ± 9	17 ± 14	7 ± 3	7 ± 6	13 ± 7
0.5-2.5 hr	8 ± 4	6 ± 3	4 ± 3	2 ± 1*	4 ± 3
7 d	7 ± 6	6 ± 4	6 ± 3	2 ± 2	3 ± 5
14 d	5 ± 2	7 ± 5	5 ± 2	3 ± 3	2 ± 1*
With Stimulus-tone					
Maximum amplitude of muscle response (mv)					
pre-dose	851 ± 601	928 ± 727	1022 ± 390	812 ± 391	708 ± 439
0.5-2.5 hr	609 ± 415	790 ± 416	770 ± 783	352 ± 276	729 ± 501
7 d	1525 ± 905	1519 ± 691	1492 ± 713	624 ± 549*	821 ± 581
14 d	1439 ± 975	1318 ± 1065	1684 ± 1144	926 ± 699	378 ± 258*
Time to reach maximum amplitude (ms)					
pre-dose	22 ± 26	20 ± 14	20 ± 25	18 ± 15	26 ± 25
0.5-2.5 hr	24 ± 32	20 ± 28	28 ± 41	37 ± 39	22 ± 31
7 d	8 ± 12	7 ± 9	6 ± 7	35 ± 24*	24 ± 28
14 d	9 ± 12	9 ± 15	16 ± 18	10 ± 11	16 ± 20
Average amplitude of muscle response (mv)					
pre-dose	119 ± 71	135 ± 62	169 ± 66	130 ± 59	110 ± 49
0.5-2.5 hr	127 ± 67	160 ± 66	130 ± 87	63 ± 44	124 ± 65
7 d	235 ± 92	253 ± 76	262 ± 97	109 ± 74*	149 ± 83
14 d	199 ± 77	214 ± 120	260 ± 125	162 ± 119	111 ± 51

[a] Data extracted from Study MRA 686-177, Table 8
[b] N = 10 for all groups except the 1200 mg/kg males for which N = 9 on days 7 and 14.

* Significantly different from control; p ≤ 0.05

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Table 5b. Auditory Response Data for Female Rats Given Technical Dicamba by Gavage[a,b]

Parameter	Auditory Response Values (mean ± S.D.) by Dose Level (mg/kg)				
	0	300	600	1200	Positive control
Control-no stimulus					
Maximum amplitude of muscle response (mv)					
pre-dose	35 ± 24	40 ± 20	28 ± 11	35 ± 30	44 ± 53
0.5-2.5 hr	26 ± 16	15 ± 5	18 ± 6	12 ± 6	13 ± 7
7 d	42 ± 25	20 ± 11*	27 ± 17	20 ± 14	8 ± 2*
14 d	23 ± 17	28 ± 14	21 ± 7	18 ± 16	8 ± 3*
Time to reach maximum amplitude (ms)					
pre-dose	55 ± 34	57 ± 29	44 ± 33	76 ± 13	55 ± 29
0.5-2.5 hr	47 ± 29	40 ± 31	55 ± 28	52 ± 34	52 ± 34
7 d	44 ± 35	46 ± 36	41 ± 27	56 ± 34	30 ± 25
14 d	38 ± 31	51 ± 35	72 ± 29	48 ± 40	48 ± 42
Average amplitude of muscle response (mv)					
pre-dose	9 ± 5	14 ± 7	9 ± 4	8 ± 7	13 ± 9
0.5-2.5 hr	5 ± 2	5 ± 2	5 ± 2	2 ± 1*	3 ± 2
7 d	9 ± 6	6 ± 4	8 ± 7	4 ± 3	2 ± 1*
14 d	6 ± 6	9 ± 5	6 ± 2	5 ± 6	2 ± 1
With Stimulus-tone					
Maximum amplitude of muscle response (mv)					
pre-dose	1096 ± 789	1144 ± 878	1359 ± 1263	661 ± 258	950 ± 573
0.5-2.5 hr	1286 ± 742	810 ± 560	938 ± 416	366 ± 181*	1008 ± 773
7 d	1049 ± 472	981 ± 552	1653 ± 883	710 ± 634	1041 ± 536
14 d	1243 ± 795	1381 ± 1004	1280 ± 780	670 ± 588	464 ± 264*
Time to reach maximum amplitude (ms)					
pre-dose	25 ± 13	22 ± 21	17 ± 15	22 ± 21	21 ± 16
0.5-2.5 hr	9 ± 10	24 ± 25	14 ± 14	17 ± 11	18 ± 27
7 d	25 ± 17	14 ± 31	14 ± 19	24 ± 25	30 ± 23
14 d	29 ± 29	8 ± 12	24 ± 19	24 ± 20	38 ± 32
Average amplitude of muscle response (mv)					
pre-dose	139 ± 73	125 ± 44	162 ± 74	121 ± 45	136 ± 51
0.5-2.5 hr	157 ± 58	151 ± 86	155 ± 61	80 ± 33*	130 ± 47
7 d	161 ± 73	157 ± 57	198 ± 66	110 ± 60	149 ± 37
14 d	158 ± 57	181 ± 64	193 ± 72	113 ± 77	95 ± 43

[a] Data extracted from Study MRA 686-177, Table 8
[b] N = 10

* Significantly different from control; $p \leq 0.05$

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Unless noted below, all effects were observed only on the day of dosing. In the home cages at 0.5-2.5 hours after dosing, rats showed slight impairment of respiration and rigidity upon handling. The incidence of these observations increased with dose. Males showed slight impairment of respiration at all doses and rigidity at 600 and 1,200 mg/kg. Females showed slight impairment of respiration at 600 and 1,200 mg/kg and rigidity at all doses.

In the open field at 0.5-2.5 hours after dosing, both male and female rats given dicamba showed a raised posture, slight-to-moderate impairment of gait, freezing when prodded, and an impaired righting reflex. Males showed decreased arousal and a statistically significant decrease in the number of rears/minute. At the lower doses, the primary effect on the righting reflex was that it appeared uncoordinated, but at higher doses, landing on the side or back were more frequently observed. Several animals became rigid upon dropping in the righting reflex test and/or the touch response test. The incidence and/or severity of these signs increased with dose. With the exception of raised posture (observed in males only at the two highest doses) these signs were observed at all doses.

In the performance testing at 0.5-2.5 hours after dosing, males showed significantly increased tail flick latency at 600 and 1,200 mg/kg and significantly decreased forelimb grip strength at all doses when tested. The decrease in forelimb grip strength was also observed at the high dose on day 7. These effects were not observed in females. Hindlimb grip strength was not significantly affected in males or females, but there was a trend toward decreasing strength with increasing dose in males. Testing for landing foot splay was impaired by the tendency of animals to become rigid upon being dropped. However, animals that were successfully tested showed no significant effects on foot splay.

Locomotor activity was significantly decreased in both males and females at the mid and high doses on the day of dosing. The locomotor activity of mid-dose animals was significantly lower than controls for the first 10 minutes of recording and remained slightly, but not significantly, lower than controls for an additional 10-15 minutes. The locomotor activity of high-dose animals was significantly lower than controls for the first 15-20 minutes of recording and slightly, but not significantly, lower than controls for an additional 10 minutes. Beyond 20 minutes of recording, the activity of controls had decreased to a level below which differences were difficult to observe, particularly in males.

Animals at the highest dose showed significant impairment in the auditory startle response. The maximum and average amplitudes of the muscle response to a tone stimulus were significantly decreased in males at the day-7 test and in females when tested on the day of dosing. These amplitudes were also slightly, but

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not significantly, lower than controls when tested at other intervals. Males also showed a significant increase in the time to reach maximum amplitude following stimulation at the day-7 test. At 1,200 mg/kg, peak muscle tone (males) and average muscle tone (males and females) during the habituation period were significantly lower than controls when tested on the day of dosing.

Many of the effects observed in rats following dosing with dicamba were not observed following acrylamide dosing. Such effects included rigidity, raised posture, decreased arousal, freezing movement when touched, increased tail flick latency, impaired righting reflex, decreased locomotor activity, impaired startle response. In addition, effects observed following dosing with either agent (i.e., impaired gait, decreased forelimb grip strength, impaired respiration) showed different time courses. The effects seen with dicamba were generally seen only on the day of dosing, whereas effects seen after acrylamide were not observed until day 7. These differences between dicamba and acrylamide indicate that the nature of the neurological changes caused by the two agents was probably different.

8. Sacrifice and Pathology

All surviving animals were sacrificed by intraperitoneal injection of sodium pentobarbital between study days 15 and 18. Prior to sacrifice, 6 rats/sex/group received a whole-body perfusion with physiological saline followed by buffered glutaraldehyde/ paraformaldehyde solution. An extra male each at 0, 600, and 1,200 mg/kg were perfused because of concerns regarding the quality of the perfusion in at least 1 male at these doses. Necropsies were conducted on all animals, including the male at 1,200 mg/kg that died on the day of dosing. Tissues marked with an "X" below were preserved in 10% neutral buffered formalin or appropriate fixative and were examined histologically in all perfused vehicle control, high-dose, and positive control animals. All tissues except the sciatic, sural, and tibial nerves were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The sciatic, sural, and tibial nerves were embedded in plastic (glycol methacrylate), sectioned, and stained with toluidine blue 0. Longitudinal sections of these nerves were stained with luxol fast blue and counterstained with periodic acid-Schiff stain. In addition, the lung, pituitary, mid-thoracic spinal cord, eyes, and anterior tibialis and gastrocnemius muscles were preserved and examined macroscopically in all animals.

Central Nervous Tissues	Peripheral Nervous Tissues
X Forebrain	X Gasserian ganglia
X Center of cerebrum	X Cervical dorsal root ganglia
X Midbrain	X Lumbar dorsal root ganglia
X Cerebellum	X Dorsal and ventral root fibers (cervical level)
X Pons	X Dorsal and ventral root fibers (lumbar level)
X Medulla oblongata	X Proximal sciatic nerve
X Lumbar spinal cord	X Sural nerve
X Cervical spinal cord	X Tibial nerve
X Lumber dorsal root ganglia	

(a) Macroscopic examination

No treatment-related effects were observed at gross necropsy in either dicamba- or acrylamide-treated rats.

(b) Microscopic examination

No treatment-related effects were observed upon histopathological examination of nervous system tissues from dicamba-treated rats. Positive control rats showed minimal-to-moderately severe axonal degeneration in the sural and tibial nerves of several animals.

B. DISCUSSION

Review of the final report and supporting data indicates that the conduct and design of the study were adequate and the reporting of the results was accurate. The data demonstrate transient (observed only on the day of dosing), dose-related effects on neurobehavioral parameters. In the home cage, the only effect apparent was a slight impairment of respiration at all doses on the day of dosing. However, when the animals were removed from the home cage and placed in an open field on the day of dosing, rats of both sexes showed rigidity in response to handling, prodding, and dropping, freezing of movement when touched, decreased arousal and fewer rears/minute compared to controls, and impairment of gait and righting reflex at all doses. Males at all doses also showed significantly decreased forelimb grip strength. At the two highest doses, locomotor activity was also significantly decreased in both sexes and males showed a raised posture and increased tail flick latency. At the highest dose, both males and females showed a decreased startle response to an auditory stimulus. By day 7 of the study, most responses had returned to control levels with the exception of the decrease in forelimb grip strength and impairment of the startle response in high-dose males which were still apparent at day 7. By day 14 of the study, no significant differences were observed between treated and control animals. Histopathological analyses of nervous system tissues showed no significant lesions.

The effects observed after dicamba exposure may be contrasted with those observed after exposure to the positive control (acrylamide). While a few effects such as impaired gait, decreased forelimb grip strength, and minimally impaired respiration were observed in both dicamba- and positive control-treated rats, many effects were not observed in both groups. For example, positive controls did not show rigidity, raised posture,

decreased arousal, freezing movement when touched, increased tail flick latency, impaired righting reflex, or decreased locomotor activity. Also, histopathological changes seen in positive control animals (minimal-to-moderately severe peripheral nerve degeneration) were not observed in dicamba-treated rats. These differences indicate that the basic neurological changes occurring in these two groups of animals may have been different. Despite these differences, the choice of acrylamide as the positive control was appropriate as this chemical is typically used to show peripheral nerve damage. Use in this study enabled the authors to conclude that no peripheral nerve damage occurred as a result of exposure to dicamba. Future tests of neurotoxicity should also include a centrally acting agent to assist in evaluating effects on central nervous system function.

The behavioral changes noted above were generally observed in the absence of significant effects on body weight or food consumption. Only high-dose males showed significant decreases in body weight, body weight gain, and food consumption. The effects on body weight gain and food consumption were observed during the first week of the study.

The LOEL for behavioral effects of dicamba is 300 mg/kg. This study satisfies the guideline requirements for an acute neurotoxicity test in rodents and is classified as Core Minimum because although a good dose-response was observed, no NOEL was determined.

END